"Analogous" Organic Synthesis of Small-Compound Libraries: Validation of Combinatorial Chemistry in Small-Molecule Synthesis

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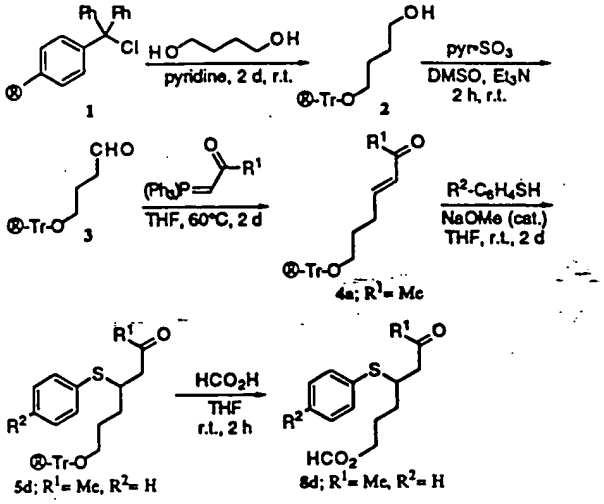
Merrifield's pioneering work in the area of polymer-supported polypeptide synthesis[2] has led to the development of polymer-supported synthesis as an important new synthetic strategy[3] in organic chemistry,[4] and the literature associated with polymer-supported reagents and catalysts is extensive.[5] Furthermore, recent interest in the preparation of large libraries of molecularly diverse compounds for deployment in various screening protocols has led to the development of a number of intriguing polymer-supported synthetic strategies.[6] This renaissance in compound screening of "combinatorial libraries" has, for the most part,[7,8] focused on chemically synthesized peptide libraries where the diversity is constrained to amide and protecting group chemistries.[9]

Intrigued by the potential of rational synthesis applied to organic (non-peptide, non-oligosaccharide, and non-nucleotide) molecule libraries, we have set out to explore and validate a number of the key issues inherent in "analogous" (i.e., solid-phase split-mix) organic synthesis.[10] *Such technology would combine the potentially limitless diversity of synthetic organic reactions and reagents with the innate advantages that small organic molecules bring to the discovery of bioavailable therapeutic agents.* In this report, we address a number of the chemical questions relevant to preparing small organic molecule libraries by targeting the analogous organic synthesis of β-mercapto ketones **8**. These substrates were selected for this investigation because collectively they illustrate (i) the potentially tremendous *reaction* versatility of analogous organic synthesis (in this case, the novel adaptation of well-documented solution-phase protection, oxidation, Horner–Emmons condensation, Michael addition, and deprotection chemistry to solid-phase split-mix chemistry) and (ii) the potentially vast *reagent* selection which can be brought to bear in analogous organic synthesis (in this case, the ready availability of ylide and thiolate reagents). Finally, targeting a limited (in this case, nine compound) library in this demonstration project has allowed us to hold this library to the normal structural characterization (^{1}H and ^{13}C NMR, LRMS, and HRMS) standards of organic chemistry.

Scheme 1



The chemistry targeted for this study was first explored as a solid-phase serial synthesis to establish the validity of each synthetic step (Scheme 1). 1,4-Butanediol was attached to the polystyrene support (● = polystyrene/2% divinyl benzene copolymer) by trityl ether[11] monoprotection (in this reaction, polymer-based site isolation[3a,12] effectively minimizes bis-protection), and the free hydroxyl of **2** was relayed to the corresponding aldehyde (**3**) by a sulfur trioxide–pyridine-mediated oxidation.[13] Horner–Emmons condensation of THF-swollen resin **3** with 1-triphenylphosphoranylidene-2-propanone delivered enone **4a** and set the stage for Michael addition of aryl thiolate. Treating resin **4a** with thiophenol and a catalytic amount of sodium methoxide delivered Michael adduct **5d**, which, upon trityl ether solvolysis with formic acid, delivered the targeted formate ester **8d**. It is noteworthy that each transformation in this scheme can be monitored by KBr pellet FT-IR analysis of the polymer.[14]

The next objective was to adapt this chemistry to a "split-mix" organic synthesis method.[15] To showcase this approach, it was decided that polymer-bound aldehyde **3** would be divided into equal portions in three separate flasks and subsequently condensed

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(1) M.J.K. is an NIH RCDA recipient (1989–1994; EC00182).

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(3) (a) Beebe, X.; Schore, N. E.; Kurth, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 10061–2. (b) Moon, H.-S.; Schore, N. E.; Kurth, M. J. *J. Org. Chem.* **1992**, *57*, 6088–9.

(4) Reviews: (a) Fréchet, J. M. J. *Tetrahedron* **1981**, *37*, 663–83. (b) Leznoff, C. C. *Acc. Chem. Res.* **1978**, *11*, 327–33. (c) Crowley, J. I.; Rapoport, H. *Acc. Chem. Res.* **1976**, *9*, 135–44.

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(8) The potential of solid-phase synthesis in the preparation of small organic molecule libraries has been discussed: Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997–8.

(9) (a) Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767–73. (b) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmiersky, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82–4. (c) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84–6. (d) Zuckermann, R. N.; Kerr, J. M.; Siani, M. A.; Banville, S. C.; Santi, D. V. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 4505–9. (e) Nikolaiev, V.; Stierandova, A.; Krchnak, V.; Seligmann, B.; Lam, K. S.; Salmon, S. E.; Lebl, M. *Pept. Res.* **1993**, *6*, 161–70. (f) Kerr, J. M.; Banville, S. C.; Zuckermann, R. N. *J. Am. Chem. Soc.* **1993**, *115*, 2529–31. (g) Nielsen, J.; Brenner, S.; Janda, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 9812–3.

(10) We propose the term "analogous organic synthesis" to set small molecule diversity apart from peptide/oligosaccharide/nucleotide diversity (e.g., these strategies being referred to as "combinatorial synthesis") as well as to convey the notion that the resulting "analogue libraries" arise from and encompass *both* reagent and chemical reaction diversity.

(11) (a) Frechet, J. M. J.; Nuyens, L. J. *Can. J. Chem.* **1976**, *54*, 926–34. (b) Fyles, T. M.; Leznoff, C. C.; Weatherston, J. *Can. J. Chem.* **1978**, *56*, 1031–41.

(12) For comment regarding the role of site isolation, see: Crowley, J. I.; Rapoport, H. *Acc. Chem. Res.* **1976**, *9*, 135–44.

(13) While oxalyl chloride/Et_3N/DMSO conditions resulted in acid-catalyzed deprotection of the trityl moiety, sulfur trioxide pyridine complex gave very satisfactory results: Parikh, J. R.; von E. Doering, W. *J. Am. Chem. Soc.* **1967**, *89*, 5505–7.

(14) (a) Functional group changes were monitored by FT-IR (KBr pressed windows of ground polystyrene beads) as follows: **1** → **2** accompanied by appearance of O–H stretch at 3572 cm^{-1}, **2** → **3** accompanied by appearance of C=O stretch at 1724 cm^{-1}, **3** → **4** accompanied by appearance of C=O stretch at 1674 cm^{-1}, and **4** → **5** accompanied by appearance of C=O stretch at 1712 cm^{-1}. (b) Frechet, J. M.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492–6.

(15) (a) See ref 9b. (b) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 487–93.

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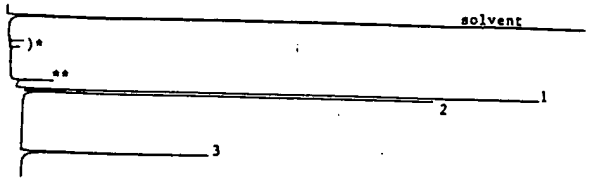
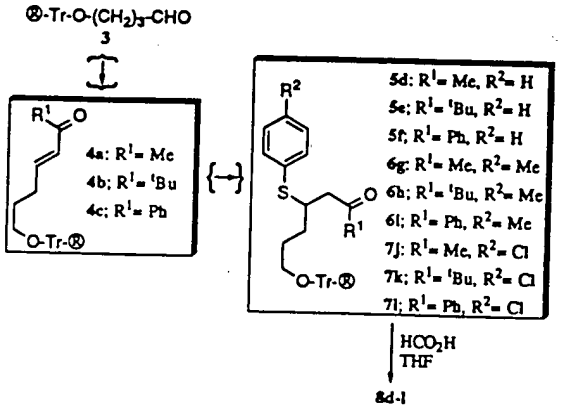


Figure 1. Capillary GC analysis (Hewlett-Packard 5890; 30-m × 0.25-μm DB210 fused silica column) of the sublibrary from flask 1 (peak no. = product/retention time/relative peak area): 1 = 8d/9.56 min/1.09, 2 = 8e/9.91 min/1.00, 3 = 8f/16.68 min/1.22 [* = unrelated impurities; ** = $HCO_2CH_2CH_2CH_2CH{=}CHC(=O)C(CH_3)_3$].

Scheme 2



with three different ylide reagents. The ylides selected for conversion 3 → 4 were the R^1 = Me/^{t}Bu/Ph analogues, and, after the separation–reaction–recombination sequence ["split-mix" method represented in Scheme 2 as {→}], a resin mixture was obtained which consisted of beads of **4a**, beads of **4b**, and beads of **4c**.

This mixture of beads was again equally divided into three flasks, the resin swollen with THF, and each flask treated with a Michael donor; flask 1 received thiophenol, flask 2 received *p*-thiocresol, and flask 3 received 4-chlorothiophenol. Prior to recombining the contents of each flask (i.e., sublibrary), small samples of resin were removed and incubated with a THF/HCO_2H mixture (1:3, 12 h)[16] to liberate the small molecule products. The THF/HCO_2H solution was withdrawn from the beads, evaporated, and the residue taken up in benzene. We were pleased to find that capillary GC analysis of each sublibrary mixture showed essentially only the three targeted formate esters **8** (i.e., formate esters from **5d–f**, **6g–i**, and **7j–l**), which were fully characterized by ^{1}H and ^{13}C NMR, HRMS, and low-resolution GC–MS.[17]

The chromatogram for the mixture obtained from flask 1 is shown in Figure 1 and illustrates that, while there were no purification steps involved in this analogous organic synthesis save bead washings between steps, the desired products are obtained in excellent purity. Chromatograms for sublibraries from flasks 2 and 3 were equally clean, establishing that each of the Horner–Emmons condensation reactions proceeds nicely, as does each Michael addition reaction. It also suggests that a deconvolution bioassay (i.e., a strategy wherein the lead compound in a library is "discovered" by successive analysis of the library and its predecessor sublibraries) requiring resin-free substrates could be utilized to screen an "analogue" library (i.e., a library of small compounds resulting from analogous organic synthesis).

(16) It is noteworthy that trityl ether solvolysis reaction conditions of 3:1 THF:HCO_2H/10 min/room temperature deliver the alcohols corresponding to **8**, 1:3 THF:HCO_2H/12 h/room temperature deliver the formate esters **8**, and intermediate conditions deliver alcohol and formate mixtures.

(17) After this manuscript was submitted, the use of GC to characterize the products of a combinatorial synthesis was reported: Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922–6.

Bioassays run on resin-bound material are also intriguing, and, in a preliminary probe of the feasibility of analogous organic syntheses utilizing this strategy, we were engaged with the question of whether compound identity could be determined on a per bead basis. This quest posed the challenge of demonstrating the feasibility of characterizing product mixtures complicated by: (i) diversity of synthetic subunits (potentially far exceeding that encountered in peptide synthesis), (ii) the small amounts of material available (≈10^{-10} mol/bead), and (iii) the potential for heterogeneity among modified polymer beads. Clean sample preparation and handling techniques coupled with sensitive GC–MS protocols were employed for this purpose. Because common substructures often yield characteristic mass spectral peaks, potential undesired side reactions as well as optimization of the analogous organic synthesis could be addressed by searching for the presence of these peaks by GC–MS.

To address this issue of per bead analysis, a single bead (200–400 mesh) was isolated with the aid of a microscope and placed in a glass melting point tube (1-mm diameter). Incubation with THF/HCO_2H (10 μL, overnight) followed by evaporation, residue dissolution in hexane (2 μL), and GC–MS analysis easily detected that single bead's formate ester product **8**. Nine random single-bead analyses of our nine-compound analogue library (pooled resins **5**, **6**, and **7**) detected the formates of **8d** (detected in three analyses), **8e**, **8g**, **8h**, **8k** (detected in two analyses), and **8l**. Clearly, single-bead analysis of 200–400 mesh resin is straightforward, with the only difficulty being mechanical issues related to handling a single small bead (≈100-μm diameter). Experiments are currently underway with 20–40 mesh beads, as these are easily manipulated with forceps without the aid of a microscope.

As a final demonstration of the reliability of analogous organic synthesis, separate 800-mg samples of sublibrary resins **5**, **6**, and **7** were incubated with THF/HCO_2H and the THF-soluble residues purified by preparative thin-layer chromatography. A UV-active band was observed for each formate ester, which was subsequently isolated and fully characterized. By this process, flask 1 delivered the formate ester derivatives **8d–f** in 27%, 11%, and 25% (respectively) overall isolated yield based on 1.57 mmol of trityl chloride/g of resin (flask 2 gave **8g–i** in 24%/17%/19%; flask 3 gave **8j–l** in 24%/7%/20%).

In summary, these results demonstrate that analogous organic synthesis using simple and universally available laboratory technology can deliver organic compound analogue libraries which appear suitable for both resin-bound and resin-free bioassays. This demonstration project utilized a 2 (number of linear synthetic steps) × 3 (number of flasks used per step) matrix and reliably delivered the nine targeted substrates. With these results in hand, we may now employ this general and expedient analogous organic synthesis methodology in bioassay-targeted libraries, and such studies targeting antioxidant compounds are currently in progress and will be reported shortly. Finally, it is noteworthy that an "analogous organic synthesis" matrix, where X_{i-n} denotes the number of flasks in analogous steps i through n, leads to an analogue library of $X_iX_{ii}X_{iii}...X_n$ compounds. Hence, analogous organic synthesis can result in analogue libraries of great diversity.

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Supplementary Material Available: Complete experimental procedures for the preparation of resins **5–7**, experimental details for the solvolysis **5–7** → **8d–l**, capillary GC chromatograms for the sublibraries, and GC–MS data for the sublibraries (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.